U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-291 458

Analysis of Polychlorinated Biphenyl (PCB) in Human Blood Serum Samples

Environmental Science and Engineering, Inc, Gainesville, FL

Prepared for

Environmental Protection Agency, Washington, DC Office of Toxic Substances

Oct 77

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA 560/6-78-007	2.	3. RECIPIENT'S ACCESSION NO. PB291458
4. TITLE AND SUBTITLE Analysis of Polychlorinated Biphenyl (PCB) in Human Blood Serum Samples		5. REPORT DATE October 1, 1977 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Charles L. Stratton and Paul C. Geiszler		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Science and Engineering, Inc. P.O. Box 13454 Gainesville, Florida 32604		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO. 68-01-3248
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Protection Agency Office of Toxic Substances 401 M Street, SW Washington, D.C. 20460		13. TYPE OF REPORT AND PERIOD COVERED Final 1/77-10/77 14. SPONSORING AGENCY CODE EPA-OTS 500/00
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

A total of 208 human blood serum samples and two mother's milk samples were analyzed for polychlorinated biphenyl (PCB). The samples were supplied to Environmental Science and Engineering, Inc. (ESE) in frozen condition by the Department of Health, Education, and Welfare, Center for Disease Control, Atlanta, Georgia. This report includes the analytical results for these samples and an assessment of the degree of uncertainty involved in the analysis.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Polychlorinated biphenyls, PCB, blood serum, analytical methods		
18. DISTRIBUTION STATEMENT Unlimited--release to public	19. SECURITY CLASS (This Report) unclassified 20. SECURITY CLASS (This page) unclassified	21. NO. OF PAGES 66 22. PRICE A04-A01

EPA Form 2220-1 (9-73)

EPA 560/6-8-007

PB 291458

ANALYSIS OF POLYCHLORINATED BIPHENYL (PCB) IN HUMAN BLOOD SERUM SAMPLES

OCTOBER 1977

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF TOXIC SUBSTANCES
WASHINGTON, D.C.

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

DISCLAIMER

This report has been reviewed by the Office of Toxic Substances, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ANALYSIS OF POLYCHLORINATED BIPHENYL (PCB) IN HUMAN BLOOD SERUM SAMPLES

Final Report
Research Request No. 2
Contract 68-01-3248

Prepared By:

Charles L. Stratton and Paul C. Geiszler

ENVIRONMENTAL SCIENCE AND ENGINEERING, INC.
P. O. Box 13454, University Station
Gainesville, Florida 32604

For:

OFFICE OF TOXIC SUBSTANCES
ENVIRONMENTAL PROTECTION AGENCY
Washington, D.C. 20460

October, 1977

CONTENTS

	Page
Figures	ii
Tables	iii
1. Introduction	1
2. Analytical Procedure	2
Quantitation	2
Confirmation	9
References	16
Appendices	
A. Analytical data results	
B. Analytical procedure	
C. Sample calculation	
D. Data for PCB pool samples	
E. Perchlorination procedure	

FIGURES

Number		Page
1	Typical Chromatogram of a Serum Extract	3
2	Chromatogram of Aroclor 1242	4
3	Chromatogram of Aroclor 1254	5

TABLES

Number		Page
1	Inter-Run Statistics for PCB Pool Samples	7
2	Inter-Laboratory Comparison of PCB Pool Sample Results	8
3	Results of Confirmatory Analysis for PCB	10
4	Reagent Blank Statistics	14
5	Comparison of Pattern versus Perchlorination Results for PCB Pool B	15

INTRODUCTION

A total of 208 human blood serum samples and two mother's milk samples were analyzed for polychlorinated biphenyl (PCB). The samples were supplied to Environmental Science and Engineering, Inc. (ESE), in frozen condition by the Department of Health, Education, and Welfare, Center for Disease Control, Atlanta, GA (CDC). This report includes the analytical results for these samples and an assessment of the degree of uncertainty involved in the analysis. The analytical results, expressed as Aroclor® 1242 and Aroclor® 1254, are tabulated in Appendix A.

ANALYTICAL PROCEDURE

The analytical procedure used is described, in detail, in Appendix B. This procedure, which is based on the procedure used by the Michigan Department of Health, was developed by the cooperation of CDC and ESE. Quality control was maintained during each sample run by:

1. Establishing and documenting chromatogram resolution by analysis of a chlorinated hydrocarbon pesticide mixture;
2. The analysis of blind duplicate samples;
3. The analysis of reagent blank samples;
4. The analysis of a chlorinated hydrocarbon pesticide mixture carried through the procedure;
5. The analysis of two serum pool samples.

QUANTITATION

Each chromatogram was quantitated as Aroclor 1242 and Aroclor 1254 as described in Appendix B. Figure 1 is a typical chromatogram of a serum sample extract. Figure 2 is a standard Aroclor 1242 chromatogram at the same gas chromatographic conditions. Figure 3 is a standard Aroclor 1254 chromatogram. Figure 1 shows the baseline used for peak height measurement. The same convention was used for the construction of the baseline on all samples. The peak heights were summed for all matching peaks (within a 4 percent retention time window) for the respective Aroclor pattern. Appendix C shows the steps of typical sample quantitation.

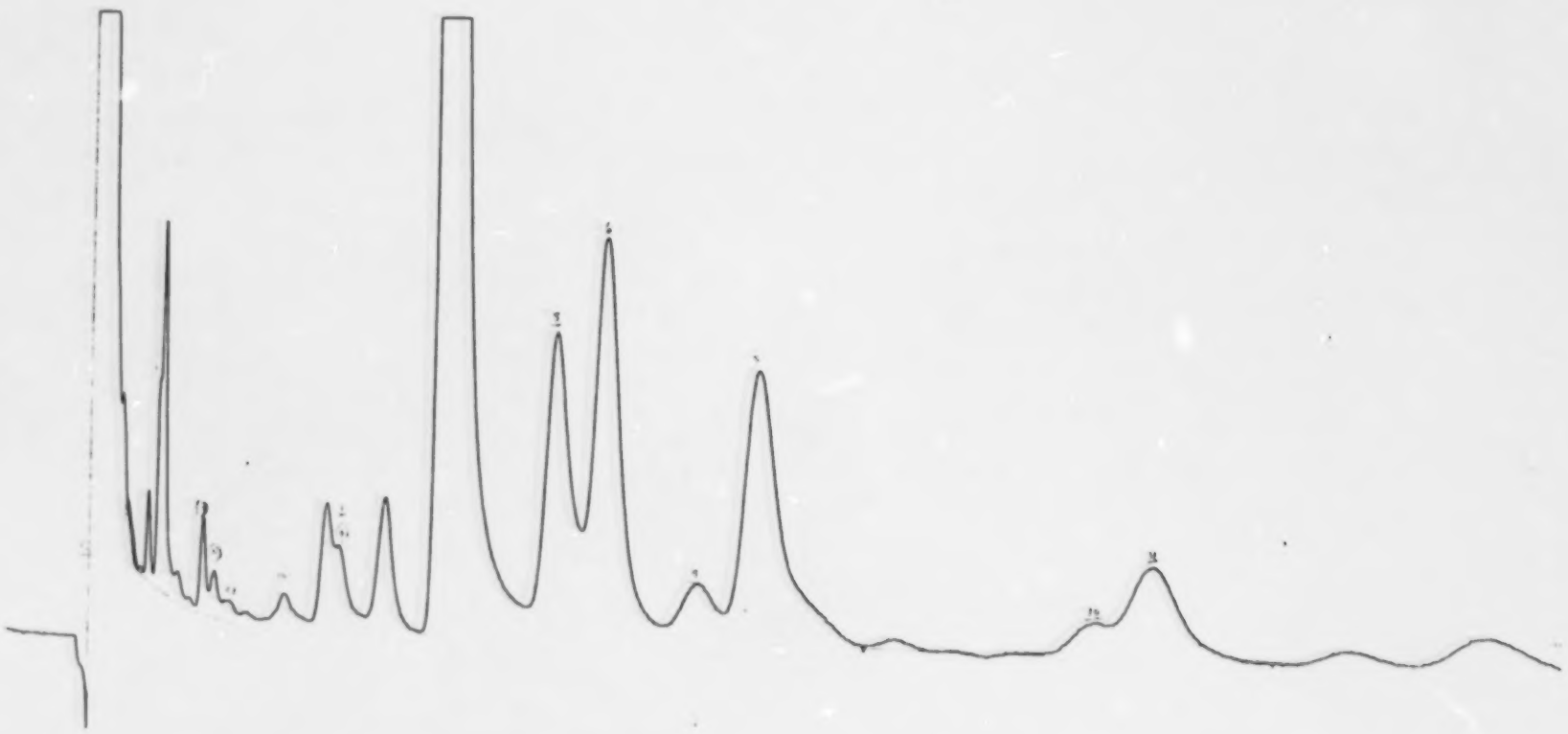


Figure 1. Typical Chromatogram of a Serum Extract

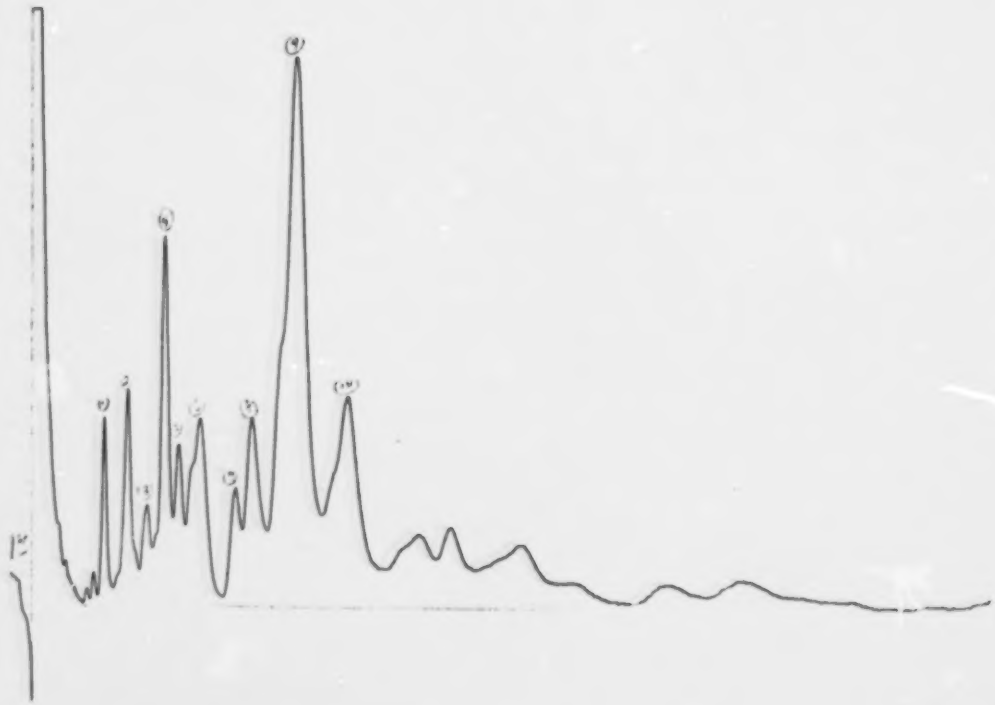


Figure 2. Chromatogram of Aroclor 1242

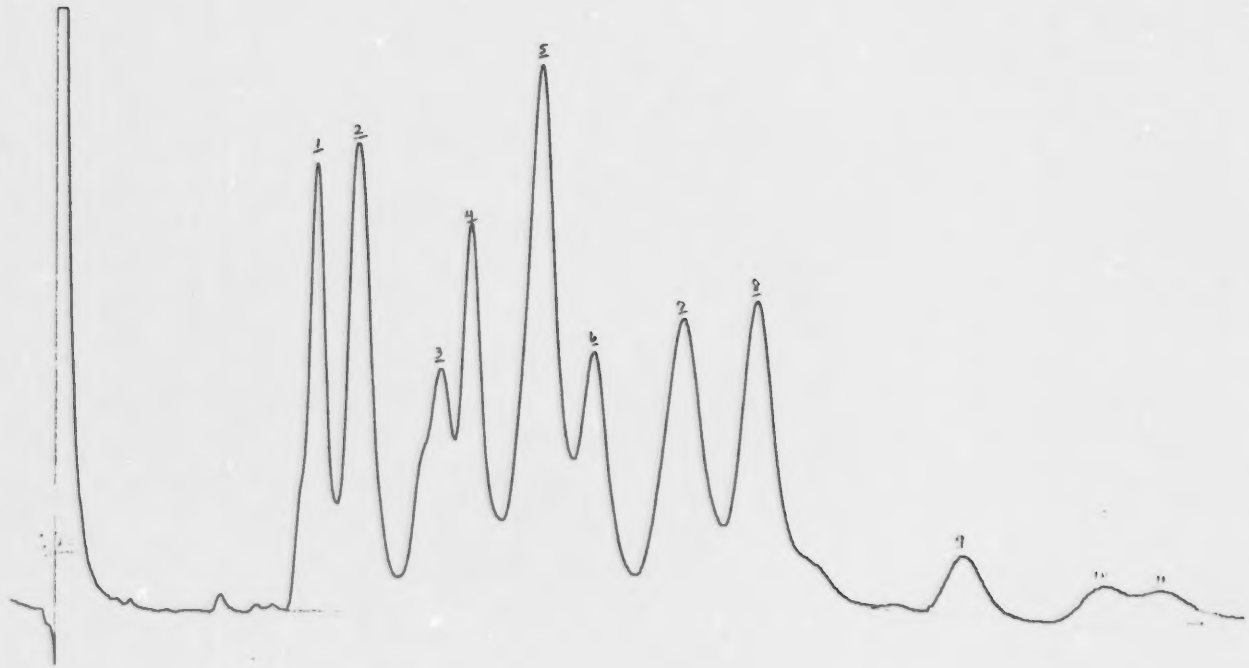


Figure 3. Chromatogram of Aroclor 1254

Each analytical run contained a PCB Pool A and a PCB Pool B sample. These pool samples consisted of serum prepared and provided by CDC. Aroclor 1242 was spiked into PCB Pool B. Appendix D is a summary of CDC analytical results for these two pool samples.

Table 1 is a summary of the inter-run statistics for the PCB pool samples as analyzed by ESE. PCB Pool A contained a mean Aroclor 1242 concentration of 6.2 ng/ml and a mean Aroclor 1254 concentration of 7.4 ng/ml using the quantitation convention described above. These low PCB levels are very near the analytical detection limit of the method. At these levels, the analytical relative standard deviation is approximately 36 percent. PCB Pool B was spiked by CDC with Aroclor 1242 to a calculated concentration of 81.1 ng/ml. The mean Aroclor 1242 concentration reported for the 26 analytical runs is 80.5 ng/ml with a relative standard deviation of 10.7 percent from run to run. The background level of Aroclor 1254 in PCB Pool B is 17.7 ng/ml with about a 30 percent relative standard deviation.

Table 2 summarizes inter-laboratory statistics for the PCB pool samples. The correspondence between CDC and ESE results for Aroclor 1242 in these pool samples is good. It should be noted in Table 2 the standard deviation of the ESE analyses reflects inter-run variability, while the standard deviation for the CDC analyses reflects intra-run variability. For PCB Pool B, the ESE mean result is 99 $\pm$ 10 percent of the Aroclor 1242 spiked amount and 92 $\pm$ 10 percent of the mean CDC result.

Table 1. Inter-Run Statistics for PCB Pool Samples

	Aroclor 1242	Aroclor 1254
PCB Pool A	$\overline{X}$ = 6.2 ng/ml	$\overline{X}$ = 7.4 ng/ml
	n = 26 runs	n = 26 runs
	σ = $\pm$ 2.4 ng/ml	σ = $\pm$ 2.5 ng/ml
	σ% = $\pm$ 38.8%	σ% = $\pm$ 33.9%
PCB Pool B	$\overline{X}$ = 80.5 ng/ml	$\overline{X}$ = 17.7 ng/ml
	n = 26 runs	n = 26 runs
	σ = $\pm$ 8.5 ng/ml	σ = $\pm$ 5.4 ng/ml
	σ% = $\pm$ 10.7%	σ% = $\pm$ 30.3%

Table 2. Inter-Laboratory Comparison of PCB Pool Sample Results

	Mean Conc. Aroclor 1242 (ng/ml)	No. of Analyses	Standard Deviation
PCB Pool A			
CDC Analysis, Aroclor 1242*	6.9	5	± 0.6
ESE Analysis, Aroclor 1242	6.2	26	± 2.4
PCB Pool B			
Spiked Aroclor 1242*	81.1	NA	NA
CDC Analysis, Aroclor 1242*	87.5	5	± 5.8
ESE Analysis, Aroclor 1242	80.5	26	± 8.5

* Data from Virlyn W. Burse memo dated May 5, 1977 (Appendix D)

CONFIRMATION

Confirmation of PCB was accomplished by perchlorination to decachlorobiphenyl (DCB) using antimony pentachloride as the derivatizing reagent as described by Armour (1973). Thirteen percent of the total number of samples were subjected to perchlorination. The perchlorination procedure is described in detail in Appendix E. Tests have shown (ESE, 1977) that this perchlorination procedure yields 100 $\pm$ 4.2 percent recovery of Aroclor standard solutions over the range of 103 ng to 10 ug.

The results of the confirmatory analyses along with the pattern matching results are tabulated in Table 3. This table shows the pattern analyzed values for Aroclor 1242 and Aroclor 1254, the calculated DCB concentration based on the pattern matching results, the analyzed DCB concentration after perchlorination of the sample, and the percent correspondence between the actual DCB value and the calculated DCB value. Conversions between Aroclor and DCB concentrations were calculated, using the factors of Armour (1973). For example, the calculation of DCB concentration in sample A0102 is as follows:

$$\frac{15 \text{ ng/ml Aroclor 1242}}{0.52} + \frac{2 \text{ ng/ml Aroclor 1254}}{0.65} = 32 \text{ ng/ml as DCB}$$

Implicit in the use of these conversion factors is the assumption that Aroclor 1242 and Aroclor 1254 are present in the serum sample in unmodified form. This is definitely not the case and, hence, the assumption is a very weak point in comparison of the pattern matching results with the perchlorination results.

Table 3. RESULTS OF CONFIRMATORY ANALYSIS FOR PCB

CDC No.	Name/Sample	Run No.	Sequence No.	Sample Vol. (ml)	Analyzed Aroclor 1242 (ng/ml)	Analyzed Aroclor 1254 (ng/ml)	Calculated DCB (ng/ml)	Analyzed DCB (ng/ml)	DCB Analyzed / DCB Calculated x 100 %
--	PCB pool B	1	9	2.5	95	21	215	166	77.2
A0102		1	2	4.3	15	2	32	18	56.3
A0103		1	13	3.6	11	5	29	15	51.7
--	PCB pool B	2	1	2.5	96	17	211	133	63.0
--	Reagent blank	3	12	5.0	0	0	0	11	--
--	PCB pool B	3	9	2.5	83	20	191	150	78.5
B0103		3	4	4.6	16	8	43	30	69.8
--	Reagent blank	4	9	5.0	4	1	10	7	--
--	PCB pool B	4	3	2.5	98	9	202	132	65.3
40-01		4	4	4.2	12	9	37	26	70.2
01-01		5	4	3.2	9	12	36	23	65.1
11-01		7	11	5.0	19	23	72	63	87.7
46-01		8	1	5.0	7	37	70	50	71.4
--	Reagent blank	8	3	5.0	0	0	0	6	--
--	PCB pool B	8	9	5.0	82	17	184	134	72.8
84-03		8	11	4.4	7	24	50	50	100.0
14-03		9	10	3.5	11	1	23	18	78.3
16-01		10	6	3.2	14	15	50	17	34.0

CDC No.	Name/Sample	Run No.	Sequence No.	Sample Vol. (ml)	Analyzed Aroclor 1242 (ng/ml)	Analyzed Aroclor 1254 (ng/ml)	Calculated DCB (ng/ml)	Analyzed DCB (ng/ml)	DCB Analyzed / DCB Calculated x 100 %
17-01		11	1	5.0	11	9	35	14	40.0
81-01		12	11	3.8	20	24	75	25	33.3
53-01		13	3	5.0	12	12	41	7	17.1
79-04		13	9	4.8	15	15	52	19	36.5
--	Nanograde hexane	--	--	5.0	0	0	0	0	--
20-03		14	5	5.0	34	29	110	37	33.6
23-02		15	6	4.2	20	26	78	16	20.5
--	PCB pool B	16	3	5.0	81	25	194	108	55.6
78-01		16	7	3.8	58	40	173	127	73.4
--	Reagent blank	16	13	5.0	2	0	4	13	--
--	PCB pool B	17	1	4.8	71	19	166	119	71.5
67-01		17	9	5.0	14	10	42	17	40.5
86-01		18	8	5.0	14	17	53	36	68.3
82-02		19	8	5.0	10	15	42	24	57.1
80-01		20	10	5.0	14	27	68	52	76.5
57-02		21	12	5.0	15	12	47	27	57.4
74-01		22	5	3.1	33	23	99	35	35.4
76-01		23	6	4.7	161	92	451	167	37.0

CDC No.	Name/Sample	Run No.	Sequence No.	Sample Vol. (ml)	Analyzed Aroclor 1242 (ng/ml)	Analyzed Aroclor 1254 (ng/ml)	Calculated DCB (ng/ml)	Analyzed DCB (ng/ml)	DCB Analyzed / DCB Calculated x 100 %
13-06		24	3	2.4	5	12	28	25	89.3
--	DCB	--	--	1.0	0	0	2140	2100	98.1
--	PCB pool B	25	1	5.[illegible]	70	18	162	133	82.1
021337		25	3	3.1	10	10	35	22	62.9
--	Aroclor 1254	--	--	0.5	0	480	738	666	90.2
--	PCB pool B	26	6	5.0	72	11	155	96	61.7
64-01		26	7	4.3	18	8	47	14	30.8
--	Reagent blank	26	10	5.0	0	1	2	2	--
021338		26	13	5.0	220	63	520	65	12.5

Reagent blank analyses were conducted during each sample run. Statistical analysis of the reported reagent blank data is presented in Table 4 for both the pattern quantitation and perchlorination data. Reagent blank values are uniformly low.

Table 5 summarizes all analytical results for PCB Pool B. The perchlorination results are 83 $\pm$ 13 percent of the CDC reported Aroclor 1242 spike, and 71 $\pm$ 12 percent of the ESE reported total Aroclor concentration.

For the 26 serum samples analyzed by both pattern matching and perchlorination, the mean analyzed DCB value is 51.4 percent of the mean calculated DCB value. The correlation coefficient between the analyzed and calculated DCB values is 0.89. Linear regression analysis yields the following empirical relationship.

$$\text{analyzed DCB (ng/ml)} = 0.38\left(\frac{\text{analyzed Aroclor 1242}}{0.52} + \frac{\text{analyzed Aroclor 1254}}{0.65}\right) + 9.0$$

Table 4. Reagent Blank Statistics

Pattern Quantitation

Aroclor 1242	Aroclor 1254
n = 36	n = 35
$\bar{X}$ = 0.6 ng/ml	$\bar{X}$ = 0.3 ng/ml
σ = $\pm$ 1.0 ng/ml	σ = $\pm$ 0.7 ng/ml

Perchlorination

DCB
n = 5
$\bar{X}$ = 7.8 ng/ml as DCB
σ = $\pm$ 4.3 ng/ml as DCB

Table 5. Comparison of Pattern versus Perchlorination Results for PCB Pool B

	PCB (ng/ml)	DCB (ng/ml)	
Spiked Aroclor 1242*	81.1	156	(c)
Pattern Analyzed Aroclor 1242 (n=26)	80.5 ± 8.5	155 ± 16	(c)
Pattern Analyzed Aroclor 1254 (n=26)	17.7 ± 5.4	27 ± 8	(c)
Perchlorination Analyzed PCB Pool B (n=9)	NA	130 ± 21	(a)

* Data from Virlyn W. Burse memo dated May 5, 1977 (Appendix D)
(a) Analytical result
(c) Calculated DCB concentration using conversion factors of Armour (1973)

REFERENCES

Armour, J. A. 1973. Quantitative Perchlorination of Polychlorinated Biphenyls as a Method for Confirmatory Residue Measurement and Identification. Journal of the Association of Official Analytical Chemists, 56(4):987-993.

Environmental Science and Engineering, Inc. 1977. Unpublished data.

APPENDIX A

ANALYTICAL RESULTS

CDC Number	Birthdate	PPM 1242	PPM 1254
A0101	---	0.009	0.002
A0102	---	0.015	0.002
A0105	---	0.008	0.002
A0106	---	0.006	0.004
A0107	---	0.011	0.007
A0108	---	0.005	0.004
A0111	---	0.005	0.007
A0112	---	0.009	0.006
A0113	---	0.011	0.005
A0114	---	0.021	0.002
A0115	---	0.011	0.005
A0116	---	0.015	0.003
A0117	---	0.007	0.002
A0118	---	0.008	0.003
A0121	---	0.002	0.003
A0122	---	0.003	0.004
A0124	---	0.007	0.003
A0125	---	0.005	0.003
B0101	---	0.007	0.001
B0102	---	0.013	0.005
B0103	---	0.016	0.008
B0204	---	0.020	0.005
B0405	---	0.011	0.017
B0406	---	0.014	0.009
39-02	7/03/38	0.003	0.003
-----	5/16/42	0.006	0.008
40-01	1/02/50	0.012	0.009
90-01	11/16/39	0.016	0.005
85-01	12/22/55	0.011	0.006
01-02	7/16/57	0.020	0.010
01-02	8/19/33	0.011	0.008
01-06	12/29/60	0.008	0.009

CDC Number	Birthdate	PPM 1242	PPM 1254
01-05	8/26/64	0.011	0.005
01-04	8/14/38	0.019	0.002
01-01	8/26/33	0.009	0.012
91-01	2/26/45	0.011	0.006
39-03	3/20/67	0.005	0.003
05-02	10/10/15	0.005	0.009
04-01	5/13/07	0.010	0.007
76-03	8/11/72	0.008	0.005
02-02	8/03/43	0.015	0.008
05-01	7/04/19	0.004	0.014
02-01	10/11/41	0.051	0.020
07-01	4/05/12	0.005	0.005
06-01	2/15/34	0.006	0.006
88-02	4/17/30	0.042	0.010
08-06	8/25/71	0.004	0.006
08-01	8/02/34	0.010	0.013
08-02	6/10/40	0.007	0.009
08-04	3/28/63	0.003	0.005
08-07	6/07/64	0.002	0.006
10-01	10/30/51	0.002	0.007
50-01	12/02/25	0.011	0.011
10-02	11/22/47	0.004	0.007
11-01	5/01/25	0.019	0.023
79-05	9/30/71	0.018	0.025
46-01	1/21/42	0.007	0.024
46-02	1/02/63	0.005	0.019
11-02	11/20/21	0.010	0.018
50-02	3/06/24	0.031	0.032
51-01	10/12/55	0.016	0.023
46-03	10/31/61	0.020	0.014
47-02	2/24/45	0.015	0.010
84-03	1/03/72	0.007	0.024

CDC Number	Birthdate	PPM 1242	PPM 1254
12-02	1/30/31	0.007	0.021
47-01	3/03/42	0.000	0.009
12-01	6/19/29	0.000	0.010
13-03	6/25/61	0.018	0.010
13-07	10/03/64	0.007	0.001
13-02	4/18/40	0.003	0.002
14-03	2/23/60	0.011	0.001
87-01	7/31/48	0.002	0.004
13-01	1/02/39	0.008	0.002
13-04	2/16/63	0.015	0.004
14-01	9/02/36	0.022	0.006
16-01	2/23/54	0.014	0.015
16-02	8/23/53	0.018	0.009
15-02	4/11/32	0.047	0.014
15-01	12/11/28	0.034	0.011
79-03	7/13/68	0.044	0.023
17-01	1/02/49	0.020	0.008
17-02	1/14/50	0.019	0.008
52-02	11/30/47	0.007	0.006
63-01	6/12/41	0.024	0.009
55-01	2/29/29	0.003	0.026
56-01	8/14/46	0.004	0.006
18-01	7/12/45	0.012	0.005
88-03	8/01/56	0.011	0.010
18-02	2/16/45	0.023	0.006
19-01	10/04/47	0.009	0.011
59-02	5/09/28	0.012	0.016
19-03	9/09/66	0.009	0.004
48-01	6/12/39	0.015	0.010
48-04	6/26/68	0.002	0.011
81-01	11/18/40	0.020	0.024
48-02	4/28/43	0.008	0.013

CDC Number	Birthdate	PPM 1242	PPM 1254
48-03	11/10/63	0.012	0.008
53-01	4/10/31	0.012	0.012
59-01	1/11/28	0.041	0.025
53-02	5/28/21	0.004	0.011
65-01	8/07/40	0.013	0.007
79-04	1/18/70	0.015	0.015
80-02	6/30/03	0.020	0.022
49-02	11/06/15	0.032	0.053
61-01	6/01/05	0.019	0.014
49-01	9/28/24	0.014	0.011
20-03	3/05/40	0.034	0.029
92-01	11/18/46	0.010	0.010
20-04	7/21/71	0.009	0.008
20-01	8/17/40	0.001	0.006
88-04	9/06/60	0.011	0.020
77-01	1/14/22	0.009	0.014
22-01	1/28/39	0.006	0.017
21-01	12/02/48	0.010	0.012
23-01	1/16/42	0.011	0.012
23-02	4/07/44	0.020	0.026
24-02	4/16/27	0.014	0.015
26-02	9/14/13	0.040	0.016
87-02	5/08/51	0.007	0.006
83-01	4/07/36	0.026	0.011
45-01	9/11/34	0.009	0.041
25-02	9/22/16	0.006	0.009
27-01	9/08/46	0.002	0.004
29-01	2/22/19	0.006	0.013
78-01	3/18/31	0.058	0.040
29-02	9/28/15	0.002	0.006
28-02	9/07/65	0.010	0.014
79-01	1/03/46	0.013	0.024

CDC Number	Birthdate	PPM 1242	PPM 1254
---	8/09/15	0.008	0.015
73-01	7/25/29	0.011	0.015
71-01	5/03/40	0.016	0.009
66-01	9/27/53	0.017	0.014
61-02	5/29/33	0.004	0.005
67-01	3/23/32	0.014	0.010
78-02	11/21/34	0.008	0.018
88-01	9/24/28	0.015	0.010
24-01	6/22/24	0.004	0.012
26-01	8/25/08	0.025	0.018
30-01	6/27/34	0.003	0.014
30-02	4/28/34	0.011	0.017
86-01	5/23/26	0.014	0.017
30-01	11/24/57	0.012	0.014
54-01	11/04/19	0.006	0.011
76-02	6/01/49	0.011	0.017
28-01	1/20/29	0.004	0.018
42-01	6/23/34	0.008	0.014
62-01	9/23/02	0.011	0.012
01-07	10/27/59	0.009	0.012
82-02	10/19/51	0.010	0.015
84-01	3/22/50	0.008	0.012
74-01	12/15/37	0.007	0.011
34-01	8/25/52	0.015	0.009
52-01	5/17/51	0.003	0.007
33-02	10/22/51	0.005	0.012
75-01	9/23/18	0.014	0.024
33-01	9/07/53	0.007	0.018
35-01	8/18/--	0.004	0.010
80-01	12/11/89	0.014	0.027
58-01	5/31/49	0.005	0.017

CDC Number	Birthdate	PPM 1242	PPM 1254
38-03	8/22/59	0.004	0.008
44-01	10/01/26	0.002	0.010
70-01	6/27/26	0.004	0.007
41-01	8/22/45	0.009	0.010
36-01	10/25/58	0.008	0.011
84-02	7/16/50	0.010	0.014
57-01	9/04/15	0.010	0.026
57-02	4/14/20	0.015	0.012
38-02	9/10/35	0.013	0.018
-----	5/07/50	0.007	0.012
38-01	2/12/43	0.012	0.011
74-01	8/25/48	0.033	0.023
68-04	4/18/24	0.012	0.007
68-02	2/24/21	0.014	0.032
79-02	2/26/47	0.020	0.022
68-01	9/14/51	0.015	0.012
68-03	3/07/45	0.003	0.009
69-01	5/07/21	0.169	0.103
43-01	10/10/51	0.084	0.032
76-01	9/27/48	0.161	0.092
41-05	---	0.004	0.009
60-05	---	0.004	0.025
02-03	---	0.005	0.004
13-05	---	0.002	0.006
13-06	---	0.005	0.012
14-05	---	0.003	0.006
16-03	---	0.004	0.003
17-03	---	0.004	0.005
19-04	---	0.004	0.008
22-02	---	0.003	0.020
22-03	---	0.003	0.013
23-03	---	0.003	0.007

CDC Number	Birthdate	PPM 1242	PPM 1254
23-04	---	0.003	0.006
38-04	---	0.004	0.013
38-06	---	0.002	0.009
41-02	---	0.003	0.007
41-03	---	0.006	0.008
41-04	---	0.000	0.008
64-01	---	0.018	0.008
60-02	---	0.006	0.007
60-03	---	0.008	0.011
-----	4/30/62	0.003	0.011
-----	10/12/55	0.003	0.012
-----	8/17/40	0.002	0.004
-----	---	0.002	0.005
021336	---	0.160	0.066
021338	---	0.220	0.063
B7	---	0.012	0.007
B8	---	0.015	0.011
B9	---	0.030	0.017
021337	1/02/50	0.010	0.010

APPENDIX B

ANALYTICAL PROCEDURE

PROCEDURE FOR

ANALYSES OF POLYCHLORINATED BIPHENYL (PCB) IN BLOOD SERUM

Prepared by:

Toxicology Branch
Clinical Chemistry Division
Bureau of Laboratories
Center for Disease Control
Atlanta, GA

and

ENVIRONMENTAL SCIENCE AND ENGINEERING, INC.
P. O. Box 13454, University Station
Gainesville, FL 32604

I. ANALYTICAL PROCEDURE

A. Extraction

1. Pipet 5 ml of serum into a 12.5 cm length culture tube to which was added 4 ml of methanol (all solvents should be of pesticide quality and stored in glass or teflon containers), attach a teflon-lined screw cap.
2. Mix the contents of the tube on a rotary mixer (50-55 rpm) for 4 minutes.
3. Add 5 ml of hexane-ethyl ether (1:1, V/V).
4. Attach the teflon-lined screw cap and place on a rotary mixer (50-55 rpm) for 15 minutes.
5. Centrifuge at 2000 rpm for 2-5 minutes.
6. Transfer the upper solvent layer by pipette to a 25 ml graduated Kuderna-Danish concentrator tube.
7. Carry out steps 3-6 twice more with fresh hexane-ethyl ether solutions; the extractions are combined in the concentrator tube.
8. Concentrate the extract to approximately 0.5 ml under a slow stream of dry organic-free nitrogen.

B. Saponification

1. Add 2 ml of a 2% (V/W methanolic potassium hydroxide solution, add an ebbulator.
2. Attach a micro-snyder column.
3. Using a Kontes Tube Heater, allow the contents to gently boil, reducing the volume to 0.3 ml.

Note: If a precipitate has formed, add a few drops of 2% methanolic KOH and warm gently in steam with swirling until the precipitate dissolves.

4. After the solution has cooled slightly, add about 2 ml of methanol-water (1:1).
5. Allow solution to reach room temperature and add 2 ml hexane into tube.
6. Stopper tube and shake vigorously.
7. Pipet out top layer.
8. Repeat steps 7-9 two additional times.

C. Column Preparation and Elution

1. Activate in a 200°C oven for 24 hours Woelm silica gel activity Grade I and granular sodium sulfate (Mallinckrodt #8024). Store in air tight flasks.
2. Deactivate the silica gel with 3% water (W/V) and allow to equilibrate at least 5 hours before use.
3. Pack a small mat of glass wool into the bottom of a 7 mm i.d. x 200 mm chromatography column, which contains a 50 ml reservoir and a teflon stopcock.
4. Mix 3g of silica gel with approximately 50 ml of hexane and pour the slurry into the column.
5. Allow the silica gel to settle and then top with a 5-7 g layer of sodium sulfate.

6. Elute the columns with 20 ml of hexane and as the last of the hexane enters the sodium sulfate layer, add the concentrated, saponified extract. Place graduated 25 ml receiver under column. Open stopcock and allow extract to just enter the sodium sulfate layer before closing stopcock.
7. Rinse the concentrator tube with 1 ml of hexane and add to the column. Open stopcock and allow rinse to just enter the sodium sulfate layer before closing stopcock.
8. Rinse the micro-Snyder-concentrator tube assembly with 2 ml of hexane and add to the column. Open stopcock and allow rinse to just enter sodium sulfate layer before closing stopcock. Note: The sodium sulfate layer should never become "dry".
9. Carefully add 25 ml hexane to the column (without disturbing the sulfate layer). Open stopcock and allow column to elute until the 25 ml receiver is filled.
10. Concentrate eluant under a slow stream of dry, organic-free nitrogen to approximately 1.0 ml.
11. Rinse the assembly by allowing 1-2 ml of hexane to run down the sides of the column.
12. The solution is then reduced to exactly 1 ml under nitrogen and is now ready for GLC analysis. Note: After analyzing by GLC it may be necessary to further concentrate or dilute this solution in order to re-analyze.

D. GLC Analysis

All analyses will be on a Varian 2700 GLC with electron capture detector. The flow rate which gives at least a 10% peak height to valley resolution between p,p'-DDE and dieldrin on the 6 ft x 1/8 in. column containing 1.5% OV-17-1.95%QF-1 should be used. The injector and detector temperature must be higher than the column which is run isothermally at 200°C. Also the operational range for the electrometer should be 10^{-10} and the attentuation should be x16 or more. All analyses must be run at the same electrometer setting; therefore, it might be necessary to dilute the sample.

E. Confirmation

A minimum of five percent of the samples will be confirmed by perchlorination to decachlorobiphenyl to insure that PCB is indeed being analyzed. The samples of the highest concentration will be selected by ESE for this analysis.

II. DESCRIPTION OF A "RUN"

A. Unknown samples and controls--to be put through the entire analytical procedure

Eight unknown samples (exception: the 27 samples from Mississippi will be in 3 runs of 9 samples each).

2. Two controls (made from human serum at CDC and designated as PCB Pool A and PCB Pool B).

3. One chlorinated hydrocarbon control containing 1 ml of the following: γ and β hexachlorocyclohexane; heptachlorepoxide; o,p'DDE; dieldrin; p,p'-DDE; o,p'DDT, p,p'-DDD and P,p'-DDT with a range of 50-100 ng/ml. This control will be prepared by ESE. When running the unknown samples, any peaks that have the same retention times as the control should not be included for quantitation.
4. Two reagent blanks, the second blank should be composed of solvents that will be used in the subsequent run to validate purity of solvents (especially useful when new solvent bottles or lot numbers are used in the next run).

B. Standards--to be analyzed by gas liquid chromatography

1. Three Aroclor 1242 standards of varying concentrations. The upper concentration should be chosen so that the tallest peak is approximately 80% full scale. Then without adjusting the attenuation, the next two concentrations should be chosen to encompass the working range.
2. Three Aroclor 1254 standards of one concentration. This concentration should be chosen such that it falls in the middle of the 1242 working concentration range.

III. INJECTION ORDER

Std 1242 (High)	Std 1254 II
Sample	Sample
Sample	Sample
Std 1254 I	Std 1242 (Medium)
Sample	Sample
Sample	Sample
Std 1242 (low)	Std 1254 III
Sample	Sample
Sample	Sample

IV. QUANTITATION

A. As 1242

Baselines for the chromatograms of the standards and the samples will be drawn by following down the sloping solvent tail and continuing out until a stable baseline is established. The retention times of all the peaks in the three 1242 standards will be measured from the solvent front to the nearest tenth of a millimeter. The three retention time values for each specific peak in the 1242 standard (i.e. from the three separate injections) are averaged and a four percent retention time window is then generated from this average retention time value. The four percent retention time window (i.e. plus and minus two percent of the retention time distance of each particular peak of the 1242 standard) is then applied for matching of sample peaks to standards.

Samples that have peaks within these windows that are offscale will be repeated to obtain all quantifiable peaks on scale. The chlorinated hydrocarbon control is gas chromatographed in the same range as the samples (i.e. if all or nearly all samples are in 1.0 ml and 5 microliters are being injected then this will be followed for the chlorinated hydrocarbon control). Peaks in the chlorinated hydrocarbon control that are within the retention time windows of the standard and are offscale in the control will be disregarded and these particular peaks will not be quantified in the standards, blanks, or in the samples since they may be attributable to pesticides in the samples and not specific PCB's. Peaks in the blank, which is analyzed in the same range as the samples, that fall in the retention time windows are measured and summed. This value is used as zero nanograms injected in the generation of a standard curve. All peaks in each of the tree Aroclor 1242 standards that have not been discarded are measured and summed and, together with the blank value, are used to generate the standard curve. The curve is established by plotting the total peak height as the Y axis versus nanograms of Aroclor 1242 injected on the X axis. The height in millimeters of all sample peaks that have not be discarded and are in the specific windows are measured and summed and the total peak height value is then plotted on the Y axis of the standard curve to give nanograms of Aroclor 1242 injected for each sample. All sample analysis points on this curve must be

between the high standard and the origin. Calculations are then carried out to give micrograms Aroclor 1242 per milliliter sample.

B. As 1254

The retention times of all the peaks in the Aroclor 1254 standards will be measured from the solvent front to the nearest tenth of a millimeter. The three retention time values for each specific peak in the Aroclor 1254 standards (i.e. one for each injection) are averaged and a four percent window is then generated from this value. Peaks in the chlorinated hydrocarbon control that are within the retention time windows of the Aroclor 1254 standards and are offscale in the control will be disregarded as was done in the Aroclor 1242 quantitation. All peaks in each of the three Aroclor 1254 standards that have not been discarded are measured and summed. The total peak height of the three separate Aroclor 1254 standards must agree within $\pm$ 10%. An average total peak height response factor is then calculated, i.e. total peak height per nanogram Aroclor 1254 injected. Sample peaks that fall within the retention time windows of the Aroclor 1254 standard, and have not been discarded must be onscale and are then measured and summed to give a total peak height of Aroclor 1254 in the samples. This value is then divided by the Aroclor 1254 response factor to give nanograms of Aroclor 1254 injected for each sample. Calculations are then carried out to give micrograms Aroclor 1254 per millimeter sample.

c. REPORTING OF RESULTS

The chromatograms will include the following information: initials of operator; date; run number; chart speed; temperature of injector; detector; and column; carrier gas and flow rate; standing current; percent full scale deflection at range of 10^{-9} and attenuation of 32; operating electrometer settings; volume of sample injected; final sample volume; and volume of serum sample. The chromatograms of the standards (1242 and 1254) will include concentration and volume injected.

VI. STORAGE OF SAMPLES

All serum samples will be stored frozen until analysis. Any unused serum sample will be stored for at least 2 months after notification of results.

VII. PREPARATION AND STORAGE OF STANDARD PCB SOLUTIONS

Aroclor stock solutions will be prepared using the Aroclor obtained directly from Monsanto Company, St. Louis, Missouri, from the following lots:

Aroclor 1016	Lot KC10-7008
Aroclor 1242	Lot KC09-415
Aroclor 1254	Lot KD05-613

Approximately 0.05 ml of the Aroclor is placed directly into a tare weighed 50 ml volumetric flask. The flask with Aroclor is then weighed and the mass of Aroclor is calculated by difference. The flask is filled to volume with nanograde 2,2,4 trimethylpentane at

20°C. This solution is shaken for a period of 4 hours and allowed to stand at least one full day to assure complete solution. This stock solution is stored at 4°C in the dark. Dilutions of this stock solution are used as Aroclor standards. All standards are stored in the dark at 4°C when not in immediate use. They are brought to room temperature and mixed before each use. Working standards should be prepared from the stock solution at least once per week.

APPENDIX C

SAMPLE CALCULATION

1242 DATA

Reproduced from best available copy.

Data for Injection #14 Run #22 5µl 1242 at 0.386 ppm = 1.93 Ng 1242 injected

1242 Peak Numbers:	①	②	③	④	⑤	⑥	⑦	⑧	⑨	omit* ⑩	Total Peak Height Peaks 1-9
Retention Time in Millimeters:	16.1	21.7	26.0	30.1	33.1	38.0	46.0	49.8	59.8	71.6	
Peak Height in Millimeters:	43	49	23	85	37	43	27	43	125	48	475

*Note: Peak ⑩ was omitted from calculations since this retention time area was offscale in the chloronated hydrocarbon control.

Retention Time Window Data Calculated from the Three 1242 Standards Injected in Run #22.

Ave of 3 standards

Peak ① Mean =	16.1 millimeters	Window is 2% either side of mean = 15.8 - 16.4 mi
Peak ② Mean =	21.7	Window is 2% either side of mean = 21.3 - 22.1
Peak ③ Mean =	26.0	Window is 2% either side of mean = 25.5 - 26.5
Peak ④ Mean =	30.0	Window is 2% either side of mean = 29.4 - 30.6
Peak ⑤ Mean =	32.9	Window is 2% either side of mean = 32.2 - 33.6
Peak ⑥ Mean =	37.8	Window is 2% either side of mean = 37.0 - 38.6
Peak ⑦ Mean =	45.8	Window is 2% either side of mean = 44.9 - 46.7
Peak ⑧ Mean =	49.7	Window is 2% either side of mean = 48.7 - 50.7
Peak ⑨ Mean =	59.7	Window is 2% either side of mean = 58.5 - 60.9
Peak ⑩ Mean =	71.4	Window is 2% either side of mean = 70.0 - 72.8

Standard Curve Data (Linear Regression) Calculated from the Three 1242 Stds

X = Nanograms 1242 injected Y = total peak height in millimeters

	X	Y
Blank	0.000	4.0
5µl 1242 at 0.1544 ppm	0.772	179.0
5µl 1242 at 0.386 ppm	1.930	475.0
2µl 1242 at 0.965 ppm	1.930	492.0

$r^2 = 0.998$
slope = 250.9
y intercept = 3.0

Data for Injection # 3 Run # 22

1242 DATA

Sample	Injection Number	Aliquot Injected	Extract Volume (ml)	Sample Volume (ml)	Peak Numbers	①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	Total Peak Height Found in Peak # ⑩	Retention Time in Seconds	Parts Per Million 1242 in Sample
					Peak Heights:	49	–	22	11	5	3	8	–	20	omit*	118	0.48	0.013
Watson, Barbara	3	4 µl	0.5 ml	4.5 ml														

* Note: Peak ⑩ Omitted because this Retention Time area (i.e. Window: 70.0 – 72.8 mm) is off-scale on the Chromatogram Hydrocarbon Fraction

1242 Calculations for Injection # 3 Run # 22

(A) This injection had peaks in its chromatogram that fell within the retention time windows for peaks ①, ③, ④, ⑤, ⑥, ⑦, and ⑨ of the 1242 standard.

(B) The peak height in millimeters for each of these peaks is given above, along with the total peak height (sum of all peaks).

(C) This total peak height of 118 mm is then plugged into the Y axis of the Standard Curve to generate an X value (i.e. how many nanograms of 1242 were actually injected when 4 µl out of 0.5 ml of the Barbara Watson Sample was injected)

X	Y
0.48 ng 1242 injected	118 mm

(D)

(E) Since 4 µl out of 0.5 ml represents 1/125th of the total extract, then 0.48 ng 1242 represents 1/125th of the total extract

(F) 0.48 × 125 = 60 ng 1242 in entire extract

(G) The extract represents the entire original sample, hence there is 60 ng 1242 in the 4.5 ml of the Barbara Watson serum.

$$\frac{60\,ng}{4.5\,ml} = \frac{0.060\,\mu g}{4.5\,ml} = \frac{0.013\,\mu g\ 1242}{ml\ serum} = 0.013\ ppm\ 1242 \text{ in the serum sample}$$

1254 DATA

Reproduced from best available copy.

DATA for INJECTION #14 RUN #22 2 µl 1254 at 0.48 ppm = 0.96 ng 1254 injection

1254 Peak Numbers:	1	2 omit*	3 omit*	4 omit*	5	6	7	8	9	10	11	Total Peak Height Excluding Peaks 2-4*
Retention Time in Millimeters:	59.5	68.7	87.5	94.5	110.3	122.3	142.5	159.0	206	238	251	
Peak Height in Millimeters:	101	106	54	87	123	58	65	70	13	8	7	445

*Note: Peaks 2, 3, 4 were omitted from the calculations since this retention time area was offscale in the chlorinated hydrocarbon control.

Retention Time Window Data Calculated From The Three 1254 Standards Injected in Run #22

	Ave. of 3 Standards	
Peak 1 Mean =	59.7 millimeters	Window is 2% either side of mean = 58.5 - 60.9 millimeters
Peak 2 Mean =	69.1	Window is 2% either side of mean = 67.7 - 70.5
Peak 3 Mean =	88.3	Window is 2% either side of mean = 86.5 - 90.1
Peak 4 Mean =	94.8	Window is 2% either side of mean = 92.9 - 96.7
Peak 5 Mean =	110.6	Window is 2% either side of mean = 108.4 - 112.8
Peak 6 Mean =	122.9	Window is 2% either side of mean = 120.4 - 125.4
Peak 7 Mean =	142.9	Window is 2% either side of mean = 140.4 - 145.8
Peak 8 Mean =	159.4	Window is 2% either side of mean = 156.2 - 162.6
Peak 9 Mean =	206.3	Window is 2% either side of mean = 202.2 - 210.4
Peak 10 Mean =	238.3	Window is 2% either side of mean = 233.5 - 243.1
Peak 11 Mean =	251.7	Window is 2% either side of mean = 246.7 - 256.7

The three separate 1254 standards made up at 0.48 ppm gave a total peak height (excluding peak 2, 3, and 4) for a 2 µl injection of:

2 µl 1254 at 0.48 ppm = 0.96 ng 1254 injected =	Total Peak Height Excluding 2-4
	448 millimeters
	444
	445
	445.7 mm average

Since 0.96 ng 1254 gives a total peak height of 445.7, then 1.0 ng 1254 should give a total peak height of 464.2 millimeters (excluding peaks 2, 3, 4)

DATA for Injection # 3 Run # 22

Sample	Injection number	µl extract injected	Dilution Volume 1	Dilution Volume 2	Total Peak Height Window 2-4 *	Nanograms 1254 injected
Watson, Barbara	3	4 µl	0.5 ml	4.5 ml	295	0.64

1254

Peak Numbers:	1	2 omit*	3 omit*	4 omit*	5	6	7	8	9	10	11
Peak Height:	20	omit*	omit*	omit*	72	95	14	64	–	8	22

*Note: Peaks 2, 3, 4, were omitted because this same retention time area (i.e. Windows: 67.7 - 70.5, 86.5 - 90.1, 92.7 - 96.7 min) were offscale in the chlorinated hydrocarbon control.

1254 Calculations for Injection 3 Run 22

Ⓐ This injection had peaks in its chromatogram that fell within the retention time windows for peaks 1, 5, 6, 7, 8, 10, 11 of

Ⓑ the peak height in millimeters for each of these peaks is given above; along with the total peak height (sum of peaks)

Ⓒ This total peak height of 295 mm is then divided by the 464.2 millimeters per nanogram 1254 injected to give nanograms 1254 injected when 4 µl out of 0.5 ml of the Barbara Watson sample was injected

$$\frac{295}{464.2} = 0.64 \text{ Nanograms 1254 injected}$$

Ⓓ Since 4 µl out of 0.5 ml represents $\frac{1}{125}^{th}$ of the total extract, then 0.64 ng 1254 represents $\frac{1}{125}^{th}$ of the total extract

0.64 ng x 125 = 80 ng 1254 in the total extract

Ⓔ The total extract represents the entire original sample, hence there is 80 ng 1254 in the 4.5 ml of the Barbara Watson serum

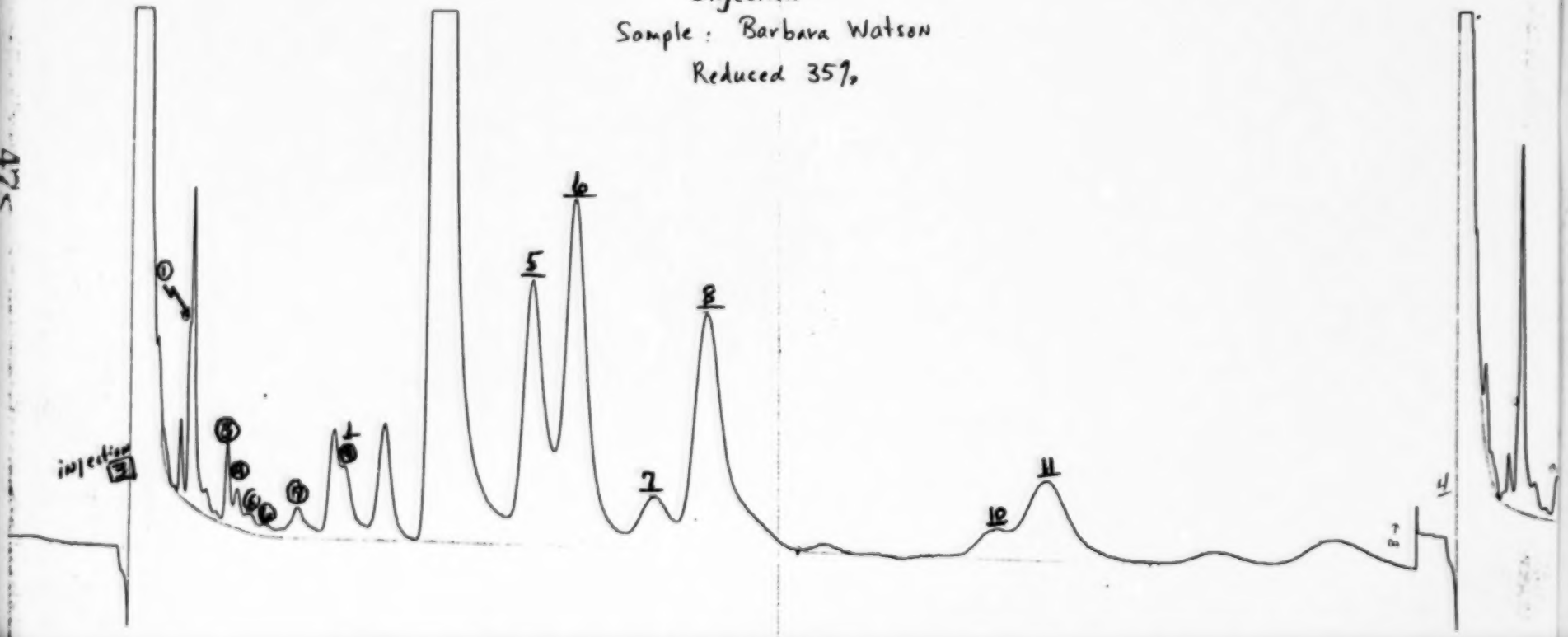
$$\frac{80 \text{ ng } 1254}{4.5 \text{ ml serum}} = \frac{0.080 \text{ ng } 1254}{4.5 \text{ ml}} = \frac{0.018 \text{ ng } 1254}{1.0 \text{ ml serum}} = 0.018 \text{ ppm } 1254 \text{ in serum}$$

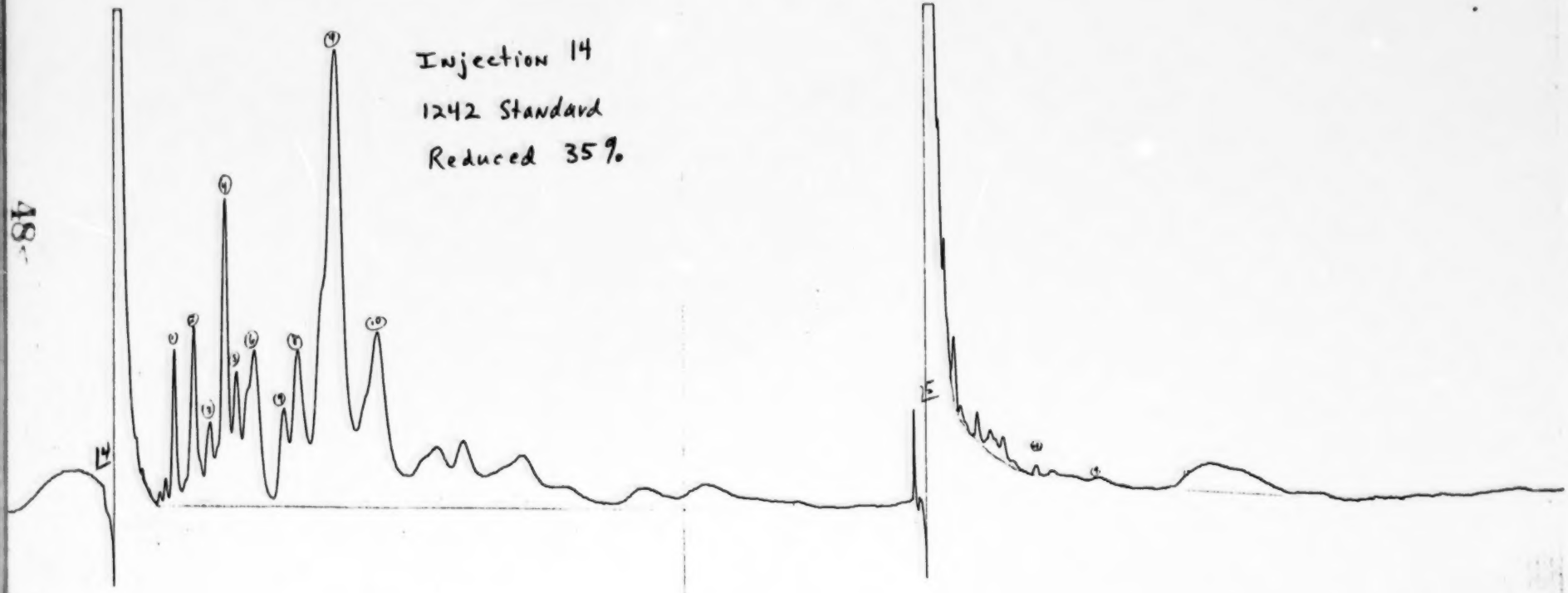
Circled Numbers (i.e. ① - ⑩) represent peaks that fall within the same Numbered peak retention time windows of the 1242 Standards

Underlined Numbers (i.e. 1 - 11) represent peaks that fall within the same Numbered peak retention time windows of the 1254 Standards

Note: Peak ⑨ of 1242 and Peak 1 of 1254 have the same retention time window and hence this peak is calculated for both 1242 and 1254

Injection 3
Sample: Barbara Watson
Reduced 35%

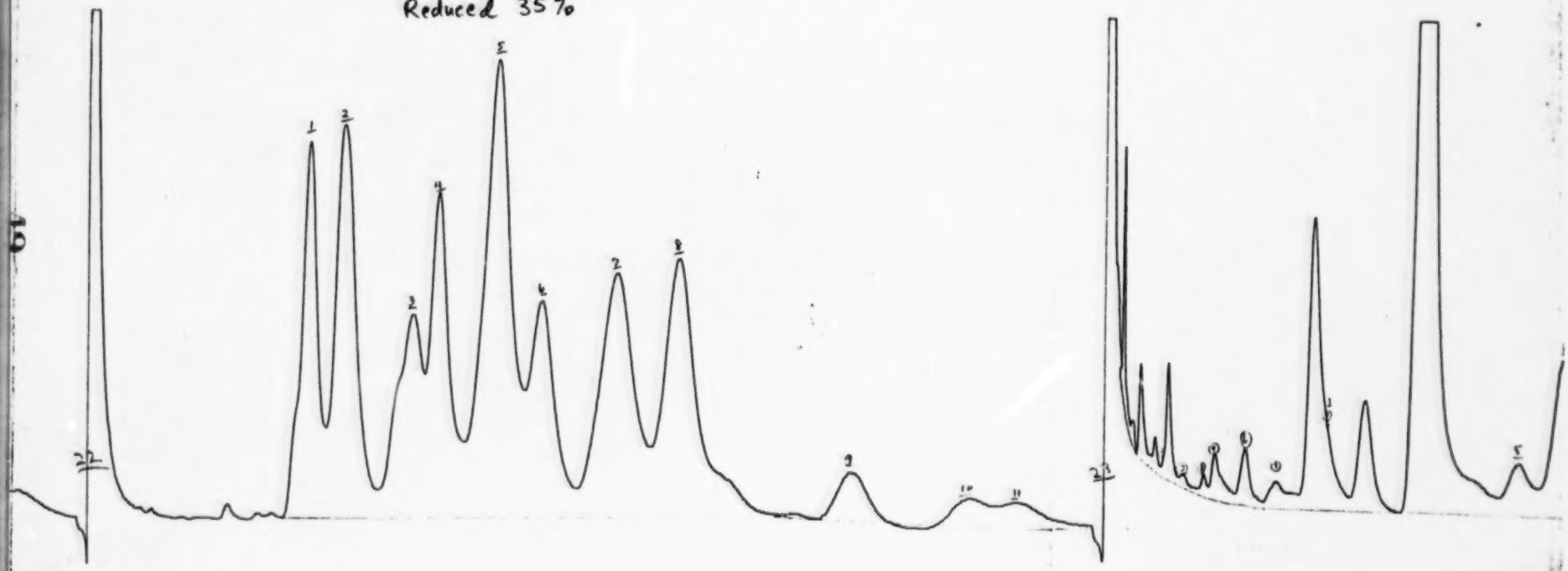



Injection 14
1242 Standard
Reduced 35%
14
48

057

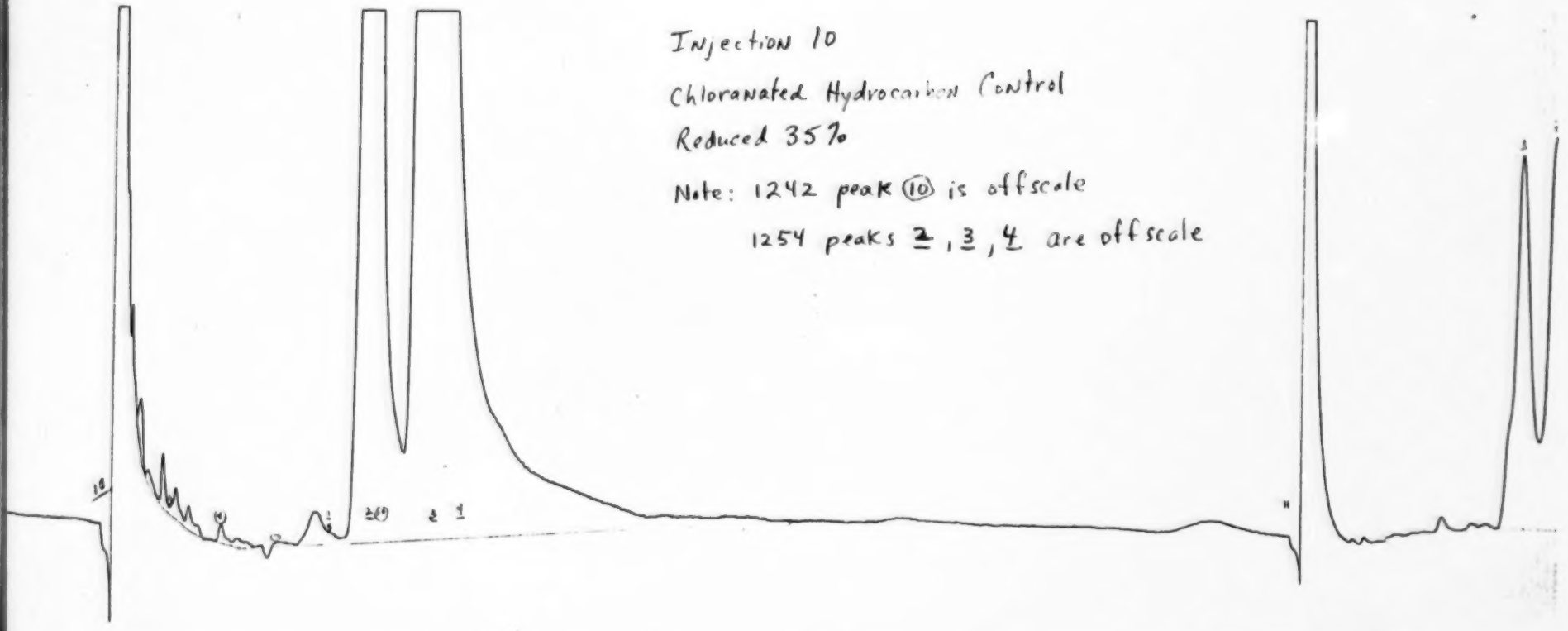
058

Injection #22
1254 Standard
Reduced 35%



50 A

042



INJECTION 10
Chloranated Hydrocarbon Control
Reduced 35%
Note: 1242 peak (10) is offscale
1254 peaks 2, 3, 4 are offscale

APPENDIX D

DATA FOR PCB POOL SAMPLES

May 5, 1977

MEMORANDUM TO THE RECORD

FROM: Virlyn W. Burse, Research Chemist, Toxicology Branch, CC, BL

SUBJ: "Characterization" of the Third ESE Pool

The purpose of this memorandum is to report data obtained by this laboratory when an attempt was made to "characterize" the third set of pools prepared for the ESE PCB project. These pools are to be used as internal bench controls by ESE once the analysis of unknowns is commenced.

In the process of determining residue levels of AR 1242, in each of the two pools, the analyst adhered to (as much as possible) those aspects of the protocol proposed for ESE (See JAL's correspondence to Charles Stratton of ESE, April 20, 1977), i.e. I.D., II.B., III and IV.A.

The values for the two pools are shown in Table 1, since they are to be viewed as two separate pools no correction was made for background of AR 1242 in the spiked pool.

The standard curve used for calculations is attached.

Virlyn W. Burse

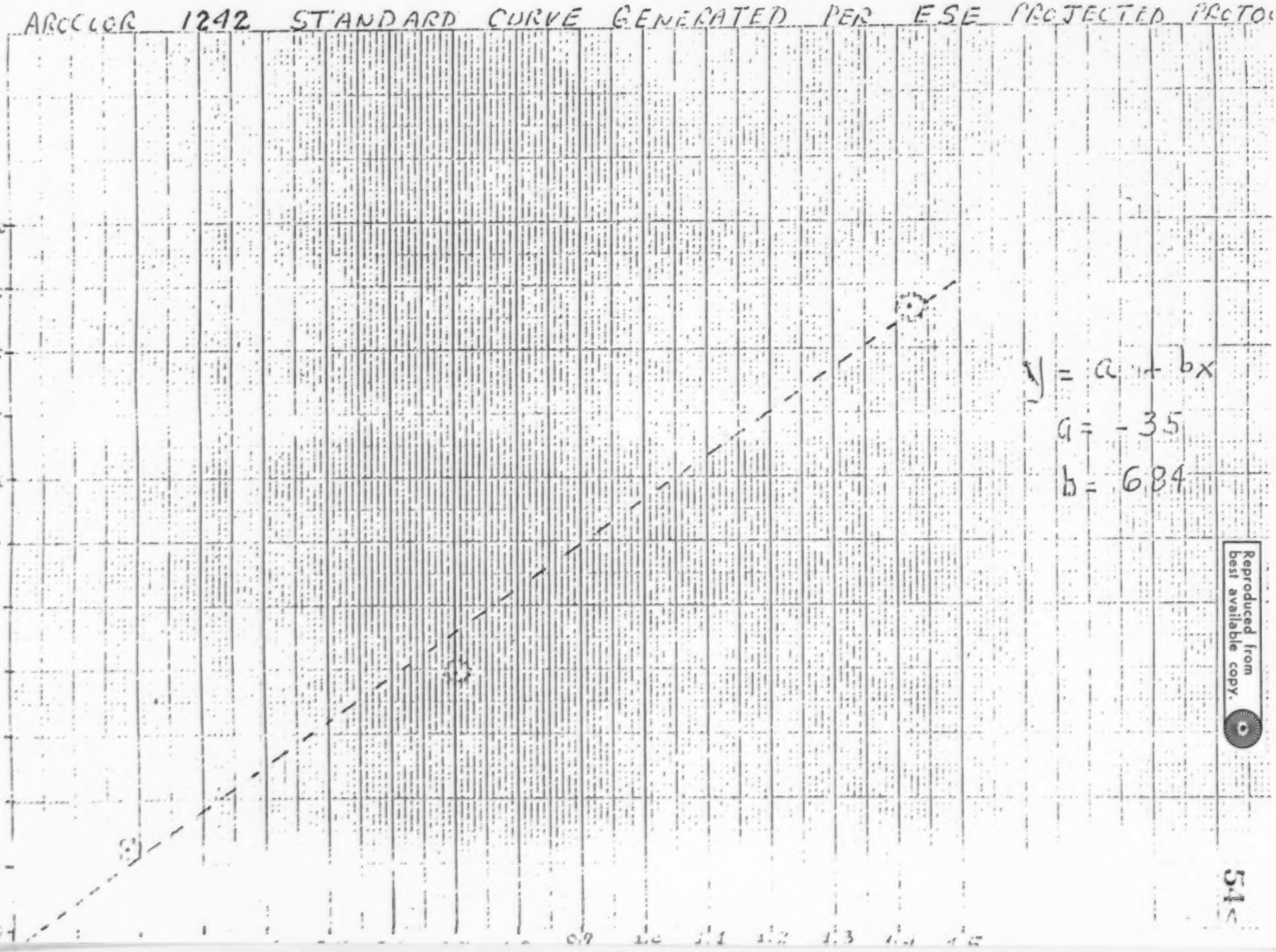
Attachment

cc: Dr. Liddle
Dr. Kimbrough
Dr. Needham

*CHARACTERIZATION OF THIRD ESE PCB POOL

Pool I.D.	Aroclor	Sample I.D.	Level of PCB in ppm	PCB Added in ppm	Prelim. Stats
ESE Project PCB Pool A	1242	A5	0.0068	None	$\bar{x}$ = 0.0069 S.D. = 0.0006 95% UCL = 0.0081 99% UCL = 0.0087 95% LCL = 0.0057 99% LCL = 0.0051
"	"	A4	0.0072	None	
"	"	A1	0.0068	None	
"	"	A3	0.0061	None	
"	"	A2	0.0077	None	
ESE Project PCB Pool B	"	B2	0.0948	0.0811	$\bar{x}$ = 0.0875 S.D. = 0.0058 95% UCL = 0.0991 99% UCL = 0.1049 95% LCL = 0.0759 99% LCL = 0.0701
"	"	B1	0.0858	"	
"	"	B4	0.0921	"	
"	"	B5	0.0816	"	
"	"	B3	0.0831	"	

*Data generated using proposed protocol approach



Reproduced from best available copy.

APPENDIX E

PERCHLORINATION PROCEDURE

PERCHLORINATION PROCEDURE FOR CONFIRMATION OF PCB

I. Principal. Perchlorination of a PCB mixture using antimony pentachloride ($SbCl_5$) as the active reagent results in conversion of all PCB present to DCB which is a single isomeric, fully-substituted species. This species has a high electron capture (EC) detector response. It can also be more readily separated from other compounds by GC techniques than can the complex PCB mixture. Quantitation can be accomplished by measurement of a single peak.

II. Sample Preparation. The sample extract (1.0 ml) prepared in the method described above is quantitatively transferred to a Kuderna-Danish apparatus with micro-Snyder column.

III. Removal of Solvent. The extracted PCB must be quantitatively exchanged from the hexane solvent to chloroform. All residual hexane must be removed from the extract prior to perchlorination. Even small amounts of residual hexane will result in the formation of a black solid residue upon the addition of $SbCl_5$. This severely reduces PCB recovery. The hexane is removed by azeotrophic evaporation from the hexane/chloroform mixture.

.1 Add 3 ml of nanograde chloroform to the Kuderna-Danish receiver containing the sample extract in hexane and concentrate, using a micro-Synder column, by slow boiling in a tube heater or water bath to about 0.2 ml. Do not allow to evaporate to dryness.

.2 Repeat step III.1 three additional times in order to remove all residual hexane. Rinse the micro-Snyder apparatus with a minimum amount of chloroform. Final volume should be approximately 1.0 ml.

.3 Quantitatively transfer to a reaction vial (Figure 1) using three chloroform rinses (Total rinse volume about 2 ml).

.4 Add two micro-Hengar boiling chips and immerse reaction vial upright in a 70°C water bath to a depth of 6 $\pm$ 2 cm.

.5 Increase water bath temperature slowly until the solvent begins to boil. Boiling temperature should be 72-76°C.

.6 Concentrate slowly to a volume of approximately 0.1 ml. Under no circumstances should the water bath temperature be permitted to exceed 76°C or the solvent be evaporated to dryness. If either of these happen, PCB will be lost by volatilization and consequent recoveries will be low. The final volume (0.1 ml) may be determined with sufficient accuracy by comparison of solvent level with another reaction vial containing 0.1 ml of chloroform.

.7 When a volume of 0.1 ml is achieved, cap the reaction vial immediately and allow to cool.

Figure 1. Perchlorination reaction vial.

IV Sample Perchlorination.

.1 To the concentrated sample extract in the reaction vial add 0.2 ml of $SbCl_5$ and immediately re-seal the vial tightly with the Teflon-lined screw cap.

.2 Place the reaction vial into a preheated (160 ± 3°C) aluminum block heater for a period of 15 hours.

.3 After the reaction period, remove the reaction vial from the aluminum block heater and allow to cool to room temperature. Then cool to 0°C in an ice water bath.

.4 Cautiously vent pressure from the vial in a fume hood, directing away from the analyst. Add 1 ml of 6 N HCl to the cool reaction vial, replae the cap tightly and shake for 30 seconds. The HCl stops the perchlorination reaction. CAUTION: IF THE REACTION VIAL IS NOT COOL, THE ADDITION OF HCl MAY CAUSE DANGEROUS SPLATTERING OF THE REAGENTS FROM THE CONTAINER.

.5 Add 1 ml hexane, shake vigorously for 30 seconds and carefully draw off the hexane layer with a disposable pipet.

.6 Place this hexane extract on the top of a 6 mm x 12 cm disposable pipet packed with 2 g of anhydrous Na_2SO_4. This column is prewashed with hexane.

.7 Repeat steps IV.5 and IV.6 five times to assure complete extraction of all DCB from the reaction vial.

.8 Pass two 1 ml portions of fresh hexane through the Na_2SO_4 column and collect all fractions in a 10 ml graduated Kuderna-Danish receiving vial.

.9 Connect a modified micro-Snyder column to the Kuderna-Danish apparatus add one Hengar boiling chip, and evaporate in a water bath (70°C) to less than 0.5 ml. Care must be taken to avoid bumping and loss of sample.

.10 Cool the apparatus to room temperature and remove the micro-Snyder column. Rinse the micro-Snyder column with sufficient fresh hexane to bring the volume up to 1.0 ml as indicated on the graduated Kuderna-Danish receiving vial. Remove the micro-Snyder column and mix by gentle swirling.

.11 Transfer the extract immediately (before significant solvent evaporation) to a properly-labeled Hypo-vial.

.12 Close the Hypo-vial with a Teflon septum and aluminum cap.

All PCB present in the sample has been converted to DCB in 1.0 ml hexane. The sample is now ready for GC analysis.

V. Analysis

.1 Analyze the DCB present in the perchlorinated extract by GC/EC. The following column conditions have been found to be suitable:

Glass column: length 6 ft., ID 1/8"
1.5% OV-17/1.95% QF-1 liquid phase
Chromosorb W-HP, 80/100 mesh support

N_2, 35 psig inlet, approximately 40 ml/min.
Column temperature--220°C
Injection port temperature--240°C
Detector Temperature--200°C

Under these conditions, DCB will elute in approximately 20 minutes.

Figure 2 is a typical chromatogram.

.2 Quantitate as DCB by comparison of the peak area with that of a known concentration of pure DCB taking into consideration all concentration factors. Care must be taken to assure the sample concentration and the standard concentration are near the same value so the EC detector is operating in the linear range. Peak areas of the standard and the sample should not differ by more than a factor of two.

$$\text{ng DCB/ml} = \frac{\frac{A - B}{S} \times C}{V}$$

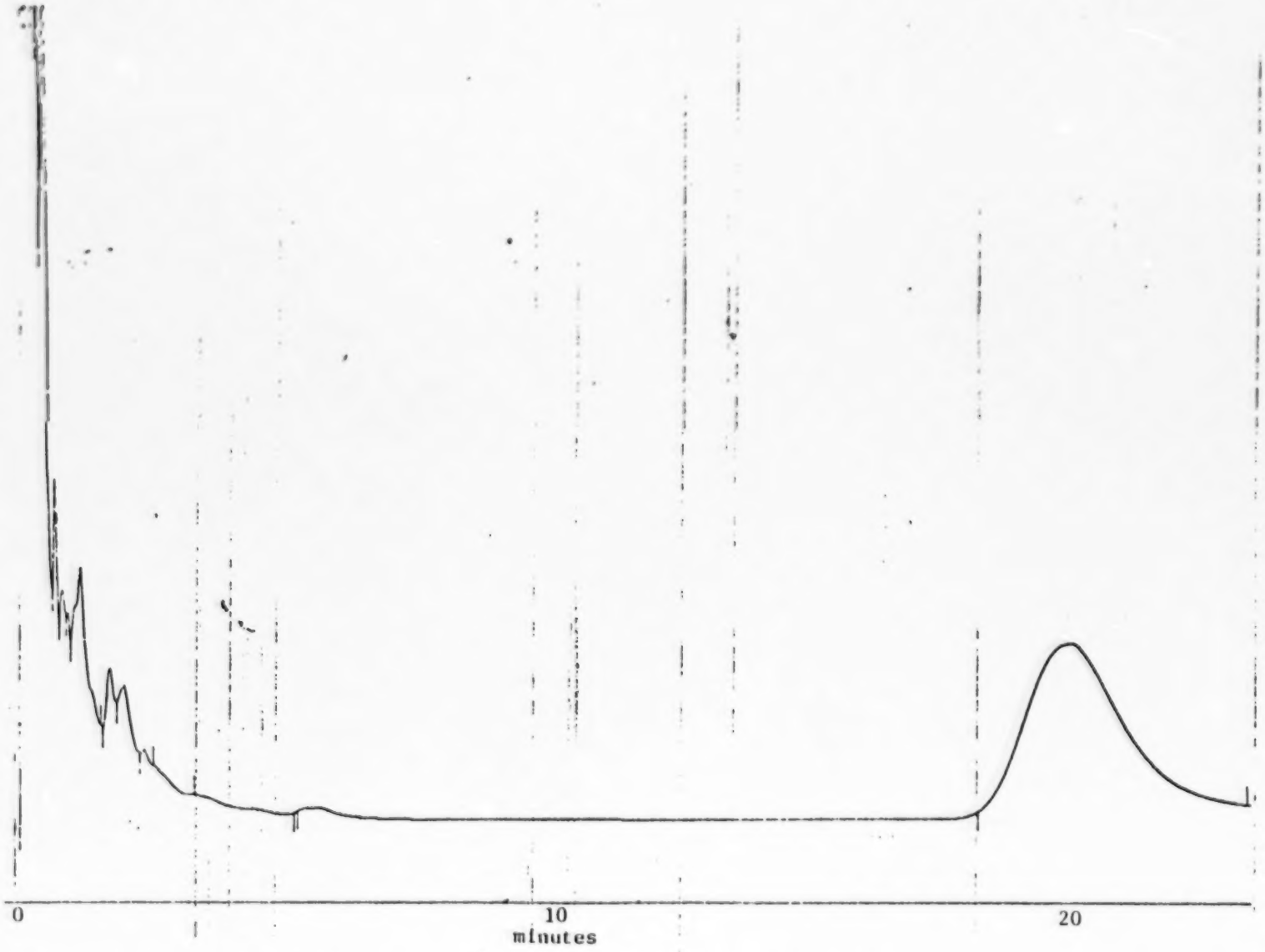


Figure 2. Typical Chromatogram Showing PCBs After Perchlorination to DCB.

where A = area of the DCB peak of the sample
B = area of the DCB peak of the procedural blank

S = area of DCB standard peak
C = concentration of the DCB standard in ng
V = volume of the sample extract

.3 To convert DCB values to approximate equivalent PCB values, Table 1 may be employed.

Table 1. Factors to Mathematically Convert Decachlorobiphenyl to an Equivalent Amount of Aroclor (5)

Aroclor	Av. No. Cl*	MW†	X**
1221	1	188.5	0.38
1232	2	223	0.45
1242	3	257.5	0.52
1016	3	257.5	0.52
1248	4	292	0.59
1254	5	326.4	0.65
1260	6	361	0.72
1262	7	395.3	0.79
DCB	10	499	1.00

* Average whole mumber of chlorines calculated from percent chlorine substitution for a specific Aroclor

† Molecular weight of Aroclor based on the average whole number of chlorines calculated from percent chlorine substitution

** X = molecular wt Aroclor/molecular wt DCB (499). To convert ppm DCB to ppm of a specific Aroclor, multiply ppm x DCB by X for the Aroclor.